

What is claimed is:

1. A protein conjugate comprising i) a physiologically active polypeptide, ii) a non-peptidic polymer, and iii) an immunoglobulin, which are covalently linked to one another, and having a prolonged *in vivo* half-life of the physiologically active polypeptide.  
5
2. The protein conjugate according to claim 1, wherein the non-peptidic polymer has two reactive groups at both ends, through which the polymer is covalently linked to the physiologically active polypeptide and the immunoglobulin.  
10
3. The protein conjugate according to claim 2, wherein the immunoglobulin is covalently linked to at least two complexes of the physiologically active polypeptide and the non-peptidic polymer.  
15
4. The protein conjugate according to claim 1, wherein the immunoglobulin is selected from the group consisting of IgG, IgA, IgD, IgE, IgM and a mixture thereof.  
20
5. The protein conjugate according to claim 4, wherein the immunoglobulin is selected from the group consisting of IgG1, IgG2, IgG3, IgG4 and a mixture thereof.
- 25 6. The protein conjugate according to claim 4, wherein the immunoglobulin is a human immunoglobulin.
7. The protein conjugate according to claim 1, wherein the immunoglobulin is selected from the group consisting of an immunoglobulin having the wild-type glycosylation, an immunoglobulin having an increased or decreased degree of glycosylation, an aglycosylated immunoglobulin and a combination thereof.  
30

8. The protein conjugate according to claim 7, wherein the increase or decrease of the degree of glycosylation or aglycosylation of an immunoglobulin is conducted by a method selected from the group consisting of a chemical method, enzymatic method, biotechnological method and a combination thereof.
9. The protein conjugate according to claim 2, wherein the reactive group of the non-peptidic polymer is selected from the group consisting of aldehyde, propion aldehyde, butyl aldehyde, maleimide and succinimide derivative.
10. The protein conjugate according to claim 9, wherein the succinimide derivative is succinimidyl propionate, succinimidyl carboxymethyl, hydroxy succinimidyl or succinimidyl carbonate.
11. The protein conjugate according to claim 9, wherein the non-peptidic polymer has aldehyde groups at both ends.
12. The protein conjugate according to claim 1, wherein the non-peptidic polymer is covalently linked at the ends thereof to the amino terminal, lysine residue, histidine residue or cysteine residue of the immunoglobulin and the amino terminal, lysine residue, histidine residue or cysteine residue of the physiologically active polypeptide, respectively.
13. The protein conjugate according to claim 1, wherein the non-peptidic polymer is selected from the group consisting of poly(ethylene glycol), poly(propylene glycol), ethylene glycol-propylene glycol copolymer, polyoxyethylated polyol, polyvinyl alcohol, polysaccharide, dextran, polyvinyl ethyl ether, poly(lactic-glycolic acid), biodegradable polymer, lipid polymer, chitin, hyaluronic acids, and a mixture thereof.
14. The protein conjugate according to claim 13, wherein the non-peptidic

polymer is poly(ethylene glycol).

15. The protein conjugate according to claim 1, wherein the physiologically active polypeptide is selected from the group consisting of hormone, cytokine, enzyme, antibody, growth factor, transcription regulatory factor, blood factor, vaccine, structural protein, ligand protein and receptor.
16. The protein conjugate according to claim 15, wherein the physiologically active polypeptide is selected from the group consisting of human growth hormone, growth hormone releasing hormone, growth hormone releasing peptide, interferons, colony stimulating factor, interleukins, glucocerebrosidase, macrophage activating factor, macrophage peptide, B cell factor, T cell factor, protein A, suppressive factor of allergy, cell necrosis glycoprotein, immunotoxin, lymphotoxin, tumor necrosis factor, tumor inhibitory factor, transforming growth factor, alpha-1 antitrypsin, albumin, apolipoprotein-E, erythropoietin, hyper-glycosylated erythropoietin, factor VII, factor VIII, factor IX, plasminogen activator, urokinase, streptokinase, protein C, C-reactive protein, renin inhibitor, collagenase inhibitor, superoxide dismutase, platelet derived growth factor, epidermal growth factor, osteogenic growth factor, osteogenesis stimulating protein, calcitonin, insulin, atriopeptin, cartilage inducing factor, connective tissue activator protein, follicle stimulating hormone, luteinizing hormone, FSH releasing hormone, nerve growth factor, parathyroid hormone, relaxin, secretin, somatomedin, insulin-like growth factor, adrenocorticotrophic hormone, glucagon, cholecystokinin, pancreatic polypeptide, gastrin releasing peptide, corticotropin releasing factor, thyroid stimulating hormone, receptor, receptor antagonist, cell surface antigen, monoclonal antibody, polyclonal antibody, antibody fragment including Fab, Fab', F(ab')<sub>2</sub>, Fd and scFv, and virus-derived vaccine antigen.
17. The protein conjugate according to claim 16, wherein the physiologically

active polypeptide is human growth hormone, interferon alpha, interferon beta, granulocyte colony stimulating factor or erythropoietin.

18. A method for preparing the protein conjugate of claim 1, comprising
- 5 (a) covalently linking at least one physiologically active polypeptide, at least one immunoglobulin with at least one non-peptidic polymer having reactive groups at both ends; and
- (b) isolating a protein conjugate comprising essentially the active polypeptide, the immunoglobulin and the non-peptidic polymer, which
- 10 are interlinked covalently.
19. The method according to claim 18, wherein step (a) further comprises:
- (a1) covalently coupling one end of the non-peptidic polymer with either an immunoglobulin or a physiologically active polypeptide;
- 15 (a2) isolating from the resulting reaction mixture a complex comprising the non-peptidic polymer coupled with the immunoglobulin or the physiologically active polypeptide; and
- (a3) covalently coupling the free end of the non-peptidic polymer of the complex with the immunoglobulin or physiologically active polypeptide,
- 20 to produce a protein conjugate comprising the physiologically active polypeptide, the non-peptidic polymer and the immunoglobulin, which are covalently interlinked.
20. The method according to claim 19, wherein the molar ratio of the
- 25 physiologically active polypeptide to the non-peptidic polymer in step (a1) ranges from 1: 2.5 to 1: 5.
21. The method according to claim 19, wherein the molar ratio of the immunoglobulin to the non-peptidic polymer in step (a1) ranges from 1: 5
- 30 to 1: 10.
22. The method according to claim 19, wherein the molar ratio of the complex

obtained in step (a2) to physiologically active polypeptide or immunoglobulin in step (a3) ranges from 1: 1 to 1: 3.

5 23. The method according to claim 19, wherein steps (a1) and (a3) are performed in the presence of a reducing agent.

24. The method according to claim 23, wherein the reducing agent is sodium cyanoborohydride, sodium borohydride, dimethylamine borate or pyridine borate.

10

25. A pharmaceutical composition having a prolonged half-life of a physiologically active polypeptide, which comprises a protein conjugate of claim 1 including i) a physiologically active polypeptide, ii) a non-peptidic polymer and iii) an immunoglobulin, and a pharmaceutically acceptable carrier.

15

26. The pharmaceutical composition according to claim 25, wherein the non-peptidic polymer has two reactive groups at both ends, through which the polymer is covalently linked to the physiologically active polypeptide and the immunoglobulin.

20

27. The pharmaceutical composition according to claim 26, wherein the immunoglobulin is covalently linked to at least two complexes of the physiologically active polypeptide and the non-peptidic polymer.

25

28. The pharmaceutical composition according to claim 25, wherein the immunoglobulin is selected from the group consisting of IgG, IgA, IgD, IgE, IgM and a mixture thereof.

30 29. The pharmaceutical composition according to claim 28, wherein the immunoglobulin is selected from the group consisting of IgG1, IgG2, IgG3, IgG4 and a mixture thereof.

30. The pharmaceutical composition according to claim 28, wherein the immunoglobulin is a human immunoglobulin.
- 5 31. The pharmaceutical composition according to claim 25, wherein the immunoglobulin is selected from the group consisting of an immunoglobulin having the wild-type glycosylation, an immunoglobulin having an increased or decreased degree of glycosylation, an aglycosylated immunoglobulin and a combination thereof.
- 10 32. The pharmaceutical composition according to claim 31, wherein the increase or decrease of the degree of glycosylation or aglycosylation of an immunoglobulin is conducted by a method selected from the group consisting of a chemical method, enzymatic method, biotechnological method and a combination thereof.
- 15 33. The pharmaceutical composition according to claim 26, wherein the reactive group of the non-peptidic polymer is selected from the group consisting of aldehyde, propion aldehyde, butyl aldehyde, maleimide and succinimide derivative.
- 20 34. The pharmaceutical composition according to claim 33, wherein the succinimide derivative is succinimidyl propionate, succinimidyl carboxymethyl, hydroxy succinimidyl or succinimidyl carbonate.
- 25 35. The pharmaceutical composition according to claim 33, wherein the non-peptidic polymer has aldehyde groups at both ends.
- 30 36. The pharmaceutical composition according to claim 25, wherein the non-peptidic polymer is covalently linked at the ends thereof to the amino terminal, lysine residue, histidine residue or cysteine residue of the immunoglobulin and the amino terminal, lysine residue, histidine residue

or cysteine residue of the physiologically active polypeptide, respectively.

37. The pharmaceutical composition according to claim 25, wherein the non-peptidic polymer is selected from the group consisting of poly(ethylene glycol), poly(propylene glycol), ethylene glycol-propylene glycol copolymer, polyoxyethylated polyol, polyvinyl alcohol, polysaccharide, dextran, polyvinyl ethyl ether, poly(lactic-glycolic acid), biodegradable polymer, lipid polymer, chitin, hyaluronic acids, and a mixture thereof.
38. The pharmaceutical composition according to claim 37, wherein the non-peptidic polymer is poly(ethylene glycol).
39. The pharmaceutical composition according to claim 25, wherein the physiologically active polypeptide is selected from the group consisting of hormone, cytokine, enzyme, antibody, growth factor, transcription regulatory factor, blood factor, vaccine, structural protein, ligand protein and receptor.
40. The pharmaceutical composition according to claim 39, wherein the physiologically active polypeptide is selected from the group consisting of human growth hormone, growth hormone releasing hormone, growth hormone releasing peptide, interferons, colony stimulating factor, interleukins, glucocerebrosidase, macrophage activating factor, macrophage peptide, B cell factor, T cell factor, protein A, suppressive factor of allergy, cell necrosis glycoprotein, immunotoxin, lymphotoxin, tumor necrosis factor, tumor inhibitory factor, transforming growth factor, alpha-1 antitrypsin, albumin, apolipoprotein-E, erythropoietin, hyperglycosylated erythropoietin, factor VII, factor VIII, factor IX, plasminogen activator, urokinase, streptokinase, protein C, C-reactive protein, renin inhibitor, collagenase inhibitor, superoxide dismutase, platelet derived growth factor, epidermal growth factor, osteogenic growth factor, osteogenesis stimulating protein, calcitonin, insulin, atriopeptin, cartilage

inducing factor, connective tissue activator protein, follicle stimulating hormone, luteinizing hormone, FSH releasing hormone, nerve growth factor, parathyroid hormone, relaxin, secretin, somatomedin, insulin-like growth factor, adrenocorticotrophic hormone, glucagon, cholecystokinin, pancreatic polypeptide, gastrin releasing peptide, corticotropin releasing factor, thyroid stimulating hormone, receptor, receptor antagonist, cell surface antigen, monoclonal antibody, polyclonal antibody, antibody fragment including Fab, Fab', F(ab')<sub>2</sub>, Fd and scFv, and virus-derived vaccine antigen.

10

41. The pharmaceutical composition according to claim 40, wherein the physiologically active polypeptide is human growth hormone, interferon alpha, interferon beta, granulocyte colony stimulating factor or erythropoietin.

15

42. A method for prolonging the *in vivo* half-life of a physiologically active polypeptide, which comprises the step of covalently linking a non-peptidic polymer having reactive groups at both ends with a physiologically active polypeptide and an immunoglobulin.

20

43. The method according to claim 42, wherein the immunoglobulin is covalently linked to at least two complexes of the physiologically active polypeptide and the non-peptidic polymer.

25

44. The method according to claim 42, wherein the immunoglobulin is selected from the group consisting of IgG1, IgG2, IgG3, IgG4, IgA, IgD, IgE, IgM and a mixture thereof.

30

45. The method according to claim 44, wherein the immunoglobulin is a human immunoglobulin.

46. The method according to claim 42, wherein the immunoglobulin is



selected from the group consisting of an immunoglobulin having the wild-type glycosylation, an immunoglobulin having an increased or decreased degree of glycosylation, an aglycosylated immunoglobulin and a combination thereof.

5

47. The method according to claim 46, wherein the increase or decrease of the degree of glycosylation or aglycosylation of an immunoglobulin is conducted by a method selected from the group consisting of a chemical method, enzymatic method, biotechnological method and a combination thereof.

10

48. The method according to claim 42, wherein the reactive group of the non-peptidic polymer is selected from the group consisting of aldehyde, propion aldehyde, maleimide and succinimide derivative.

15

49. The method according to claim 42, wherein the non-peptidic polymer is selected from the group consisting of poly(ethylene glycol), poly(propylene glycol), ethylene glycol-propylene glycol copolymer, polyoxyethylated polyol, polyvinyl alcohol, polysaccharide, dextran, polyvinyl ethyl ether, biodegradable polymer, lipid polymer, chitin, hyaluronic acids, and a mixture thereof.

20

50. The method according to claim 49, wherein the non-peptidic polymer is poly(ethylene glycol).

25

51. The method according to claim 42, wherein the physiologically active polypeptide is selected from the group consisting of hormone, cytokine, enzyme, antibody, growth factor, transcription regulatory factor, blood factor, vaccine, structural protein, ligand protein and receptor.

30

52. The method according to claim 51, wherein the physiologically active polypeptide is selected from the group consisting of human growth

hormone, growth hormone releasing hormone, growth hormone releasing peptide, interferons, colony stimulating factor, interleukins, glucocerebrosidase, macrophage activating factor, macrophage peptide, B cell factor, T cell factor, protein A, suppressive factor of allergy, cell  
5 necrosis glycoprotein, immunotoxin, lymphotoxin, tumor necrosis factor, tumor inhibitory factor, transforming growth factor, alpha-1 antitrypsin, albumin, apolipoprotein-E, erythropoietin, hyper-glycosylated erythropoietin, factor VII, factor VIII, factor IX, plasminogen activator, urokinase, streptokinase, protein C, C-reactive protein, renin inhibitor,  
10 collagenase inhibitor, superoxide dismutase, leptin, platelet derived growth factor, epidermal growth factor, osteogenic growth factor, osteogenesis stimulating protein, calcitonin, insulin, atriopeptin, cartilage inducing factor, connective tissue activator protein, follicle stimulating hormone, luteinizing hormone, FSH releasing hormone, nerve growth factor,  
15 parathyroid hormone, relaxin, secretin, somatomedin, insulin-like growth factor, adrenocorticotrophic hormone, glucagon, cholecystokinin, pancreatic polypeptide, gastrin releasing peptide, corticotropin releasing factor, thyroid stimulating hormone, receptor, receptor antagonist, cell surface antigen, monoclonal antibody, polyclonal antibody, antibody  
20 fragment including Fab, Fab', F(ab')<sub>2</sub>, Fd and scFv, and virus-derived vaccine antigen.

53. The method according to claim 51, wherein the physiologically active polypeptide is human growth hormone, interferon alpha, interferon beta,  
25 granulocyte colony stimulating factor or erythropoietin.